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Zinc-Induced Formation of a Coactivator Complex Containing the Zinc-Sensing Transcription Factor MTF-1, p300/CBP, and Sp1▽

Yong Li,[1] Tomoki Kimura,[1]‡ Ryan W. Huyck,[2] John H. Laity,[2] and Glen K. Andrews[1]*

Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, Kansas 66160-7421,[1] and Division of Cell Biology and Biophysics, School of Biological Sciences, University of Missouri—Kansas City, Kansas City, Missouri 64110-2499[2]

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Herein, the mechanisms of transactivation of gene expression by mouse metal response element-binding transcription factor 1 (MTF-1) were investigated. Evidence obtained from coimmunoprecipitation assays revealed that exposure of the cells to zinc resulted in the rapid formation of a multiprotein complex containing MTF-1, the histone acetyltransferase p300/CBP, and the transcription factor Sp1. Down-regulation of endogenous p300 expression by small interfering RNA transfection significantly decreased zinc-dependent metallothionein I (*MT-I*) gene transcription without altering induction of zinc transporter 1 (*ZnT1*). MTF-1 independently facilitated the recruitment of Sp1 and p300 to the protein complex in response to zinc. Mutagenesis demonstrated that the acidic domain, one of three transactivation domains of MTF-1, is required for recruitment of p300 but not Sp1 as well as for zinc-dependent activation of *MT-I* gene transcription. Furthermore, mutation of leucine residues (L→A) within a nuclear exclusion signal in the MTF-1 acidic domain impaired recruitment of p300 and zinc-dependent activation of the *MT-I* gene. Nuclear magnetic resonance characterization of an isolated protein fragment corresponding to the MTF-1 acidic region demonstrated that this region is largely unstructured in the presence and absence of excess stoichiometric amounts of zinc. This suggests that the mechanism by which MTF-1 recruits p300 to this complex involves extrinsic-zinc-dependent steps. These studies reveal a novel zinc-responsive mechanism requiring an acidic region of MTF-1 that functions as a nuclear exclusion signal as well as participating in formation of a coactivator complex essential for transactivation of *MT-I* gene expression.

Metal response element (MRE)-binding transcription factor 1 (MTF-1) activates metallothionein (*MT*) gene expression in response to several stresses, including exposure to metal ions (zinc and cadmium), hypoxia, oxidative stress, and elevated temperature (3, 25). Central to the mechanism of action of vertebrate MTF-1 on *MT* genes is its ability to function as a zinc sensor, and an increase in available zinc may be a unifying theme during many stresses. However, mouse MTF-1 is also essential during embryonic development of the liver (41), and this transcription factor integrates a diverse set of environmental signals and modulates the expression of many genes (26). MTF-1 interacts with or cooperates with a diverse set of factors, including NF-κB (8), hypoxia-inducible factor 1α (HIF-1α) (28, 29), USF (4), Sp1 (30), heat shock transcription factor 1 (34, 38), and ribosomal protein S35 (1).

Studies of the mechanism by which MTF-1 regulates transcription of vertebrate *MT-I* genes in response to zinc suggest that the reversible binding of zinc directly with the unique, highly conserved six-zinc-finger domain regulates the affinity of binding of the fingers to short, *cis*-acting DNA sequences, termed MREs, found in multiple copies in the *MT-I* (and other) gene promoters. Increased available zinc in the cell, resulting from rapid uptake or the redistribution of intracellular zinc, is thought to induce canonical structure in the MTF-1 zinc fingers (31) mitigated by a subset of interfinger linker peptides (24) that is accompanied by nuclear translocation, binding to the *MT-I* gene promoter, and enhanced transcription of the mouse *MT-I* gene (reviewed in references 3 and 20). In addition to the zinc finger domain, mouse and human MTF-1 proteins have domains that modulate nuclear localization (33) as well as several *trans*-activation domains (acidic rich, proline rich, serine/threonine rich [25], and cysteine rich [7]) that participate in the mechanism of action of MTF-1.

In vivo footprinting and chromatin immunoprecipitation (ChIP) assays indicate that several factors, including USF, c-Jun, and Sp1, are constitutively bound to the proximal 230 bp of the mouse *MT-I* promoter, whereas MTF-1 binding to the five MREs is induced by zinc (2). During zinc induction, the proximal 250 bp of the *MT-I* promoter becomes almost completely bound by these transcription factors (10). Whether these factors directly interact with MTF-1 is unknown. The transcriptional activities of many transcription factors involve the coactivator and histone acetyltransferase CREB binding protein (CBP) and its close relative p300 (6). CBP and p300 are encoded on separate genes but have very similar polypeptide sequences. p300/CBP is a large protein that, in addition to histone acetyltransferase activity, contains multiple regions that mediate protein-protein interactions (6). It is recruited to target gene promoters through interactions with transcription factors where it connects them to the basal transcription machinery, directs the assembly of multiprotein complexes that

* Corresponding author. Mailing address: Department of Biochemistry and Molecular Biology, Mail Stop 3030, University of Kansas Medical Center, 39th and Rainbow Blvd., Kansas City, KS 66160-7421. Phone: (913) 588-6935. Fax: (913) 588-3920. E-mail: gandrews@kumc.edu.

‡ Present address: Department of Toxicology, Faculty of Pharmaceutical Sciences, Setsunan University, 45-1 Nagaotoge-cho, Hirakata, Osaka 573-0101, Japan.

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regulate transcription, and functions as an acetyltransferase that targets chromatin and/or transcription factors to facilitate transcription (6, 39).

Herein, results are presented which indicate that zinc activation of *MT-I* transcription involves the formation of a multiprotein complex containing, at minimum, p300/CBP and Sp1. The formation of this complex is dependent upon specific leucine residues within an intrinsically unstructured acidic domain of MTF-1 which are also required for nuclear exclusion of the protein. Although the detailed mechanistic steps remain to be determined, this newly revealed metal-dependent step in the zinc-sensing mechanism of MTF-1 appears to involve a competition between nuclear exclusion and transcriptional activation.

MATERIALS AND METHODS

Materials. Antibodies against Flag peptide and protein G-agarose were purchased from Sigma. Polyclonal antibodies against p300, CBP, and hemagglutinin (HA) were purchased from Santa Cruz. Polyclonal antibody against Sp1 was purchased from Upstate Biotechnology. TRIzol RNA extraction reagent, Lipofectamine 2000, hygromycin B, and the plasmid vector pcDNA3.1/Hygro (+) were purchased from Invitrogen Life Technologies. Enhanced chemiluminescence plus reagents were purchased from Amersham Bioscience. The coupled transcription/translation reticulocyte lysate system (TnT) was obtained from Promega.

Cell culture and transfection. Mouse embryo fibroblast (MEF) cells prepared from wild-type and MTF-1 knockout (MTF-KO) mice were transfected and selected as described previously (18, 24). MEF cells were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS). MTF-KO MEF cells were transfected with pcDNA3.1/hygromycin (+) vectors that express epitope-tagged mouse MTF-1 as delineated below, using Lipofectamine 2000 reagent according to the manufacturer's instructions. Individual hygromycin-resistant colonies were isolated after drug selection, and expression was confirmed by Western blotting using anti-Flag or anti-HA antibodies. Stably transfected cell lines were maintained in DMEM supplemented with 10% FBS and 200 μg/ml hygromycin B.

Cloning, expression, and purification. The MTF-1_{flag} expression construct and methods of mutagenesis have been described previously (18, 24). MTF-1 mutants were constructed using PCR amplification followed by subcloning into pcDNA3.1/Hygro (+) and confirmed by DNA sequencing. Expression vectors for C- or N-terminally truncated MTF-1 peptides flanked by HA and Flag tags were prepared. The vector 937-C encoded amino acids 313 to 675, the vector 1210-C encoded amino acids 404 to 675, and the vector N-936 encoded amino acids 1 to 312. In addition, a vector encoding MTF-1_{flag} with the acidic domain (amino acids 329 to 405) deleted (MTF-ΔAD) and the MTF-1 acidic domain with the N-terminal green fluorescent protein (GFP) tag were prepared (see Fig. 5).

To produce the MTF-1 acidic domain, a cDNA fragment corresponding to residues 287 to 405 of MTF-1, which encompasses the region from zinc finger 6 (zf6; residues 288 to 312) to the acidic domain (residues 329 to 405), was amplified by PCR. Plasmid p21MTFzf6-405 was constructed using an approach parallel to that described previously for the MTF-1 DNA-binding domain (31, 32). Plasmid DNA sequences were verified by sequencing.

Plasmid p21MTFzf6-405 was transfected into *Escherichia coli* strain BL21(DE3) and plated, from which a single colony was used to inoculate 20 ml of Luria-Bertani broth containing 200 μg/ml ampicillin. After overnight growth, cells were gently centrifuged and resuspended in 1 liter of minimal media (1.28% Na_2HPO_4, 0.3% KH_2PO_4, 0.05% NaCl, 1 mM $MgCl_2$, and 1 mM $CaCl_2$) supplemented with 50 μg/ml ampicillin, trace elements, and 2 g/liter uniformly labeled (U) $^{15}NH_4Cl$ and 2 g/liter [U-^{13}C]glucose for nuclear magnetic resonance (NMR) studies. The cell culture was grown at 37°C until the visible absorbance at 600 nm reached 0.8, at which time expression was induced using 1 mM isopropyl-1-thio-β-D-galactopyranoside and growth continued for 3 h. Cells were pelleted via centrifugation and resuspended in sonication lysis buffer (25 mM MOPS [morpholinepropanesulfonic acid], pH 7, 300 mM NaCl, 50 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride). After lysis by sonication, soluble and insoluble fractions were separated by centrifugation at 18,000 rpm for 30 min (Sorvall SS-34 rotor). The insoluble fraction which contained the recombinant zf6-acidic domain protein was solubilized with 7 M guanidinium hydrochloride and purified by reverse-phase high-performance liquid chromatography as described previously (31). After lyophilization, protein molecular weight and purity (>95%) were analyzed by mass spectrometry and sodium dodecyl sulfate-polyacrylamide gel electrophoresis, respectively.

NMR spectroscopy. Lyophilized zf6-acidic domain protein was solubilized to 0.43 mM in argon-saturated 2-(*N*-morpholine)ethanesulfonic acidD_{13}, pH 6.9, with 5% D_2O, 5 mM β-mercaptoethanol, 0.2 mM 3-(trimethylsilyl)-1-propanesulfonic acid, and 1 mM NaN_3. The NMR sample was sealed with an airtight cap. Double- and triple-resonance spectra were acquired for the protein sample by using a 14.1 T Varian Inova spectrometer (599.7 MHz for ^{1}H, 150.8 MHz for ^{13}C, and 60.8 MHz for ^{15}N) equipped with a (^{1}H, ^{13}C, and ^{15}N) triple resonance probe at 30°C. Specifically, ^{1}H-^{15}N-HSQC, HNCACB, CBCA(CO)NH, HNCA, and HNCO spectra were recorded using standard solvent suppression methods as described previously (31) to obtain three nearly complete sets of backbone ($^1H^N$, $^{15}N^H$, and ^{13}C) and side chain ^{13}Cβ resonance assignments of the zf6-acidic domain protein: (i) with no zinc present, (ii) with 1.1 molar equivalents of Zn(II), and (iii) with 3.3 equivalents of Zn(II). NMR data were processed and analyzed using the NMRPipe (13) and NMR View (19) software packages, respectively. Residue backbone atom chemical shift values were compared to the random coil values (42, 43) for the same residue atoms by using the BioMagResBank tab2bmrb software package (V. Jaravine, Basal University).

Preparation of whole-cell, nuclear, and cytoplasmic extracts. Whole-cell, cytoplasmic, and nuclear extracts from cultured cells were prepared as previously described (24, 35). MEF cells were incubated for up to 1 h in medium containing $ZnSO_4$ (100 μM) or $CdCl_2$ (20 μM). Protein concentrations were determined using the Bio-Rad protein assay reagent, with bovine serum albumin as the standard.

In vitro transcription/translation of MTF-1. MTF-1_{flag} and MTF-ΔAD were synthesized in vitro using the TnT System, as described previously (5). Protein synthesis was monitored by Western blotting for Flag, as described below.

Immunoprecipitation and Western blotting. About 500 μg of whole-cell extract protein was used for immunoprecipitation in HEPES buffer (20 mM HEPES at pH 7.9, 150 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM dithiothreitol, and protease inhibitors). The whole-cell extracts were precleared with protein G-agarose for 1 h at 4°C. The precleared supernatants were then incubated with specific antibody for 1 to 2 h at 4°C, and immune complexes were collected by addition of protein G-agarose for 2 h at 4°C. After washing of the beads with immunoprecipitation buffer five times, the immunoprecipitated proteins were eluted, separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis gel, and analyzed by Western blotting using the indicated antibodies.

EMSA. An electrophoretic mobility shift assay (EMSA) was performed using whole-cell extracts or aliquots from the TnT lysate reactions and an end-labeled, double-stranded oligonucleotide called MRE-d under conditions described previously (5, 24).

Northern blot detection of *MT-I* and *ZnT1* mRNA. Transfected or control MTF-KO MEF cells were incubated in medium containing 100 μM $ZnSO_4$ or 10 μM $CdCl_2$ for 6 h prior to harvest. Total RNA, isolated using TRIzol reagent, was separated by denaturing agarose-formaldehyde gel electrophoresis, transferred, UV cross-linked to nylon membranes, and hybridized with an *MT-I* cRNA or *ZnT1* cRNA probes, as previously described (21, 24). Hybrids were detected by autoradiography at −70°C with intensifying screens. A β-actin cRNA probe was used as a control to normalize for RNA amount, integrity, and transfer efficiency.

ChIP assay. ChIP assays were carried out as described previously (10, 24). Transfected and control MTF-KO MEF cells were incubated in DMEM plus 10% FBS containing $ZnSO_4$ (100 μM) or $CdCl_2$ (20 μM). Cells were fixed in 1% formaldehyde; sheared chromatin was prepared, precleared with protein G-agarose, and immunoprecipitated with specific antibody overnight at 4°C. Immune complexes were captured using protein G-agarose, and the formaldehyde cross-links in the eluted complexes were reversed. The DNA was analyzed by PCR using primers that span bp −264 to +43 (relative to the transcription start site) of the mouse *MT-I* gene. PCR products (28 cycles) were separated by electrophoresis, stained with Sybr green I, quantified using the ChemiImager system, and normalized to input products. For initial experiments, input and immunoprecipitated DNA samples were analyzed at various cycles (27 to 33 cycles) to ensure that the PCR products were obtained in the linear range of amplification.

siRNA knockdown experiments. MTF-KO MEF cells or cells stably transfected with MTF-1_{flag} were plated on 24-well plates at 30 to 50% confluence overnight and then transfected with 50 μM small interfering RNA (siRNA) oligonucleotides by using Lipofectamine 2000 according to the manufacturer's instructions. The siRNA duplexes were obtained from Ambion. The sequences of siRNA used here were as follows: p300, GGUAUGAUGAACAGUCCAGtt

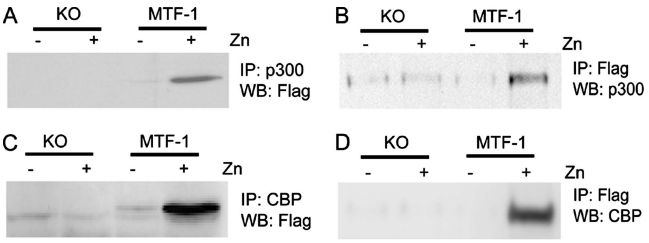

FIG. 1. Coimmunoprecipitation of mouse MTF-1 and p300/CBP in zinc-treated cells. MEF cells that lack MTF-1 (KO) or that express MTF-1_{flag} (MTF-1) were incubated for 1 h in medium with (+) or without (−) 100 μM $ZnSO_4$ (Zn). Whole-cell extracts were immunoprecipitated (IP) with an antibody against Flag (B and D), p300 (A), or CBP (C), followed by Western blot (WB) analysis of the immunoprecipitates by use of the indicated antibody.

(lowercase indicates the terminal residues that protect siRNA from degradation), and CBP, CCAAGGAGACUCAUCAACAtt. Silencer negative-control no. 1 siRNA from Ambion was used as a negative control in the experiments. Cells were harvested 72 h after transfection. Endogenous CBP and p300 levels were determined by Western blotting. Total RNA was isolated using TRIzol reagent and used for Northern blotting (2 μg) as described above or for reverse transcription-PCR (RT-PCR) (0.5 μg) with the AccuScript high-fidelity RT-PCR system according to the manufacturer's instructions. The primers used in RT-PCR were as follows: mMT-1 fwd, ATG GAC CCC AAC TGC TCC TG; mMT-1 rvs, GCGCCTTTGCAGACACAGC; β-actin fwd, GGAATGGGTCA GAAGGACTC; β-actin rvs, GTTGGCCTTAGGGTTCAGG; ZnT1fwd, ACA TGCTGTCGGACGTGCTG; and ZnT1rvs, CGACGCTCAGCACCACGAG.

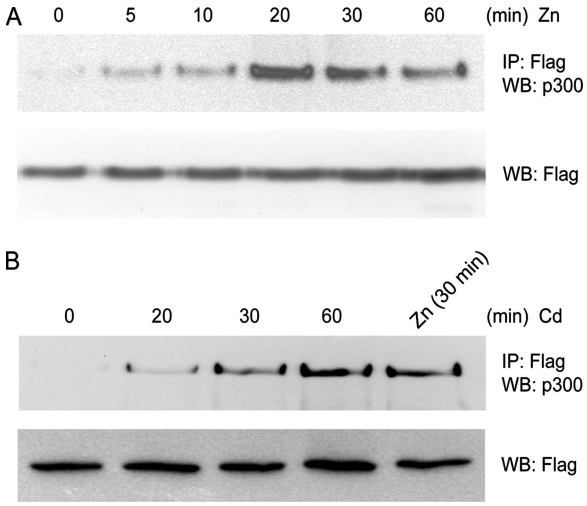

FIG. 2. Time dependence of coimmunoprecipitation of mouse MTF-1 and p300 after zinc or cadmium treatment. MEF cells that express MTF-1_{flag} were incubated for the indicated times in culture medium containing 100 μM $ZnSO_4$ (A) or 20 μM $CdCl_2$ (B). Whole-cell extracts were immunoprecipitated (IP) using an antibody against Flag. Immunoprecipitates were analyzed by Western blotting (WB), using an antibody against p300 (upper panels) or an antibody against Flag (lower panels) as a control for the efficiency of the immunoprecipitation of MTF-1 in each sample.

RESULTS

MTF-1 interacts with p300/CBP in response to zinc or cadmium. MEF cells which lack endogenous MTF-1 (MTF-KO) do not activate *MT-I* gene expression in response to zinc or cadmium. However, the zinc/cadmium responsiveness of this gene is restored in a cell line stably transfected with an expression vector encoding mouse MTF-1_{flag} (24). These cells were incubated for 1 h in medium containing zinc. Whole-cell extracts were prepared, and antibodies were used in a reciprocal coimmunoprecipitation assay for p300, CBP, and MTF-1_{flag}. Immunoprecipitated proteins were then examined by Western blotting (Fig. 1). MTF-1_{flag} coprecipitated with CBP and p300 antibodies, and these proteins coprecipitated with Flag antibodies in transfected cells treated with zinc but not in untreated cells or cells lacking MTF-1 (KO), regardless of zinc treatment (Fig. 1). These data indicate that MTF-1 forms a complex with p300/CBP in vivo in response to zinc. This finding was supported and extended in a time course study which revealed that MTF-1_{flag} could be coprecipitated with p300 within 5 to 10 min after the addition of zinc (Fig. 2A) or within 20 min after the addition of cadmium (Fig. 2B).

MTF-1 is essential for formation of a complex containing Sp1 and p300 in response to zinc. USF-1, USF-2, and Sp1 have been implicated in the regulation of mouse *MT-I* gene expression (4, 10, 30). Binding of USF-1 and USF-2 to an E-box sequence (bp −220) and a composite USF/ARE element (bp −89 to −101) is constitutive in the mouse *MT-I* promoter (10). There are also two GC-rich sites in the proximal promoter: one at bp −183 that is constitutively occupied by Sp1 and the other overlapping MRE-d (10).

In initial experiments, it was noted that Flag antibodies do not coprecipitate USF-1 in control or metal-treated, transfected cells (data not shown). In contrast, Flag antibodies and Sp1 antibodies (Fig. 3A) showed reciprocal coimmunoprecipitation of Sp1 and MTF-1_{flag} by 20 min after exposure to zinc or cadmium (Fig. 3A and B).

Formation of a complex containing Sp1 and p300 in response to zinc was detected in cells that express MTF-1 but not in cells devoid of it (Fig. 3C). Thus, MTF-1 facilitates the association of p300 and Sp1, and zinc increases the amount of the MTF-1–p300–Sp1-containing complex (Fig. 3C).

p300 plays an important role in MTF-1 activation of *MT-I* gene transcription in response to zinc. To elucidate potential functions of p300 and CBP in MTF-1 activation of *MT-I* gene expression in response to zinc, siRNAs were utilized to specifically deplete endogenous p300 or CBP in MEF-KO cells and MEF cells stably transfected with an expression vector encoding mouse MTF-1_{flag}. Cells were transfected with siRNA against mouse p300 or CBP or a nonspecific siRNA, and the abundances of these proteins were examined by Western blotting (Fig. 4A). The relative abundances of p300 and CBP were significantly reduced (but not eliminated) by their respective siRNAs 3 days after the initial transfection. This did not occur after transfection with a nonspecific siRNA control, and the relative abundance of MTF-1 was not altered by the p300/CBP siRNA transfections (Fig. 4A). Nor did p300 RNA interference (RNAi) affect CBP abundance or vice versa (Fig. 4A). The effects of depletion of p300 or CBP on *MT-I* induction in response to zinc was examined by RT-PCR (Fig. 4B) and Northern blotting (Fig. 4C). Cells were treated with zinc for 6 h, and total RNA was analyzed. The results show that depletion of p300 but not CBP clearly attenuated zinc induction of *MT-I*. Reduction of CBP was not reproducibly as dramatic as that of p300, which may account for the lack of effect of RNAi on zinc induction of *MT-I*.

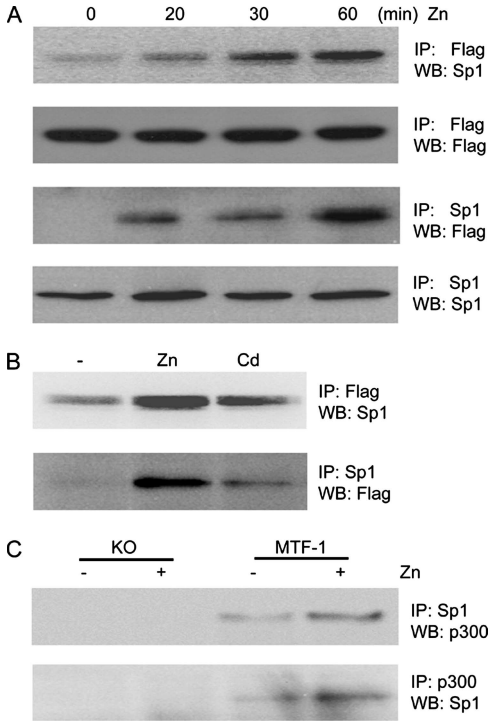

FIG. 3. Coimmunoprecipitation of mouse MTF-1 with p300 and Sp1 from zinc- or cadmium-treated cells. MEF cells that express MTF-1_{flag} were incubated for the indicated times in culture medium containing 100 μM $ZnSO_4$ or 20 μM $CdCl_2$. (A) Whole-cell extracts were immunoprecipitated (IP) using an antibody against Flag (Flag) or Sp1 (Sp1) as indicated. The precipitates were immunoblotted (WB) using an anti-Sp1 antibody or anti-Flag antibody as indicated. (B) After 1 h of incubation with zinc (Zn) or cadmium (Cd) and reciprocal coimmunoprecipitation, experiments were performed as described for panel A. (C) MEF cells that lack MTF-1 (KO) or that express MTF-1_{flag} (MTF-1) were incubated with 100 μM $ZnSO_4$ (Zn) for 1 h, and reciprocal coimmunoprecipitation experiments were performed using Sp1 or a p300 antibodies as indicated.

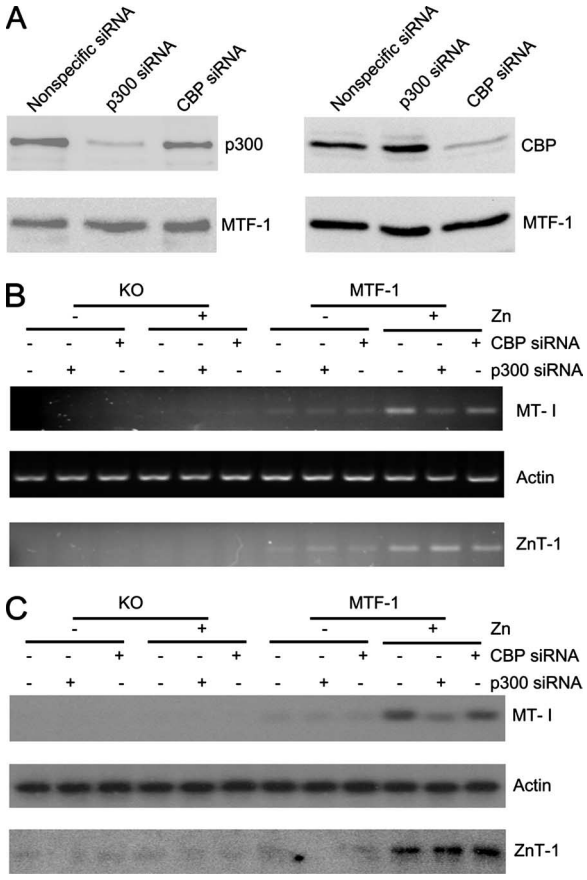

FIG. 4. siRNA knockdown of endogenous p300, but not CBP, abolishes MTF-1 induction of *MT-I* expression in response to zinc. MEF cells that lack MTF-1 (KO) or that express MTF-1_{flag} (MTF-1) were examined. (A) MTF-1 cells were transfected with siRNAs that specifically target mouse p300 or CBP or with nonspecific siRNAs, as indicated. After 3 days, whole-cell extracts were immunoblotted for p300 (left), CBP (right), and MTF-1 (lower panels). (B and C) KO and MTF-1 cells were transfected (+) or not transfected (−) with the indicated siRNA, and 3 days later they were incubated with 100 μM $ZnSO_4$ (Zn) for 6 h, total RNA was isolated, and RT-PCR (B) and Northern blotting (C) were used to examine mRNAs for *MT-I* (upper), β-*actin* (middle), and *ZnT1* (lower).

It is interesting to note that induction of the zinc efflux transporter *ZnT1*, which is also an MTF-1-responsive gene (21), was not affected by depletion of either p300 or CBP. This suggests that MTF-1 utilizes promoter-specific transcriptional complexes.

Formation of a zinc-dependent MTF-1 complex containing p300/CBP requires the acidic transactivation domain. To examine which domains of MTF-1 are critical for formation of a zinc-dependent complex containing p300/CBP, tagged-deletion mutants of MTF-1 were expressed in transfected MEF-KO cells (schematized in Fig. 5A) and coimmunoprecipitation experiments were performed using whole-cell extracts from control and zinc-treated cells (Fig. 5B to F). An MTF-1_{flag} peptide extending from just after the zinc finger domain to the C terminus (937-C; amino acids 313 to 675) coprecipitated with p300 in zinc-treated cells (Fig. 5B), whereas a similar peptide in which the acidic domain had been removed (1210-C; amino acids 404 to 675) did not. This result was confirmed by a reciprocal coimmunoprecipitation experiment using p300 antibody for immunoprecipitation and Flag antibody for Western blotting (Fig. 5C). No interaction was detected between p300 and an MTF-1_{flag} peptide extending from the amino terminus to the six-zinc-finger domain (N-936; amino acids 1 to 312) (Fig. 5D). Specific deletion of the acidic domain from MTF-1 (MTF-ΔAD) eliminated zinc-induced binding of p300 (Fig. 5E). Furthermore, the acidic domain alone attached to GFP (GFP-AD) was coprecipitated with p300 in the cells exposed to zinc (Fig. 5F). These data suggested that the acidic domain of MTF-1 plays a key role in formation of a zinc-dependent complex containing p300.

Sp1 recruitment to the zinc-dependent MTF-1 complex involves both N-terminal and C-terminal regions of MTF-1. p300/CBP and Sp1 are both components of a zinc-dependent MTF-1 complex; therefore, it was interesting to examine which domains of MTF-1 are critical for association of Sp1. Cells expressing deletion mutants of MTF-1, which are described above (Fig. 5A), were treated with zinc, and reciprocal coimmunoprecipitation experiments were used to examine Sp1 and MTF-1 peptides (Fig. 6A and B). Sp1 coprecipitated with two peptides of MTF-1: the peptide from the N terminus to the

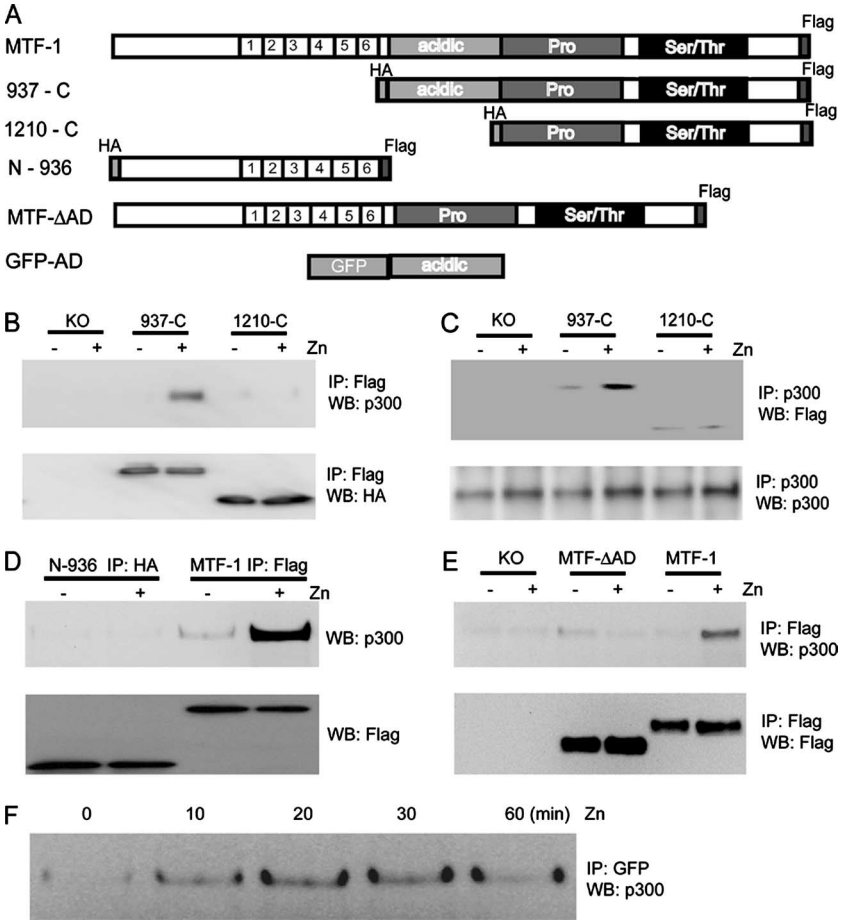

FIG. 5. Mapping the domains of MTF-1 that interact with p300 in vivo using a coimmunoprecipitation assay. MEF cells that lack MTF-1 (KO) were transfected with expression vectors encoding GFP-, HA-, and/or Flag-tagged MTF-1 peptides shown diagrammatically in panel A. The vector nomenclature refers to the nucleotide position within the coding region of mouse MTF-1 (2,025 nucleotides; 675 amino acids) and the C or N terminus of the protein. The peptides encoded were as follows: 937-C, amino acids 313 to 675; 1210-C, amino acids 404 to 675; and N-936, amino acids 1 to 312. MTF-ΔAD refers to $MTF\text{-}1_{flag}$ with the acidic transactivation domain (amino acids 329 to 405) deleted. GFP-AD refers to the MTF-1 acidic domain with an N-terminal GFP tag. (B to F) KO cells transfected with the indicated expression vectors were incubated in medium with (+) or without (−) 100 μM $ZnSO_4$ (Zn) for 1 h or the indicated times (F). Whole-cell extracts were prepared and analyzed by coimmunoprecipitation (IP) and Western blotting (WB) using the indicated antibodies (GFP, Flag, HA, or p300). Controls for the efficiency of immunoprecipitation in each set of samples are shown in the bottom panels except in panel F.

six-zinc-finger domain and the peptide from the zinc finger domain to the C terminus. Thus, recruitment of Sp1 to the zinc-dependent MTF-1 complex involves, in part, a region of MTF-1 different from that required for recruitment of p300/CBP.

The acidic domain of MTF-1 is involved in the association of p300 with an MTF-1 complex, and p300 can interact with Sp1 (16, 17, 37). Therefore, the role of the acidic domain in the recruitment of Sp1 to the MTF-1 complex was examined by coimmunoprecipitation (Fig. 6C). Deletion of the acidic domain of MTF-1 did not significantly diminish the zinc-induced association with Sp1 (Fig. 6C). These results show that recruitment of Sp1 to the MTF-1 complex is independent of recruitment of p300 and involves both N-terminal and C-terminal regions of MTF-1.

The acidic transactivation domain of MTF-1 is required for induction of *MT-I* expression in response to metals. The function of the acidic domain of MTF-1 was examined given its role in the formation of the zinc-dependent complex containing p300. Transfection experiments revealed that deletion of the acidic domain eliminated metal induction of *MT-I* mRNA (Fig. 7A). These results did not reflect the expression levels of these proteins, as shown by the Western blotting.

The zinc-dependent MRE-binding activity of MTF-1 was not diminished by deletion of the activation domain, as measured by EMSA (Fig. 7B), nor was MTF-1 binding to the *MT-I* promoter, as measured by ChIP analysis (Fig. 7C). Thus, the impaired ability MTF-ΔAD to activate *MT-I* expression in response to zinc likely reflects a loss of transactivation function and the inability to form a competent transcriptional complex containing p300. The majority of MTF-1 was constitutively localized in the nucleus after deletion of the acidic domain (Fig. 7D, bottom).

Leucine residues in the acidic domain are important for MTF-1 induction of *MT-I* expression and recruitment of p300 to a zinc-dependent complex. The role of the acidic domain of

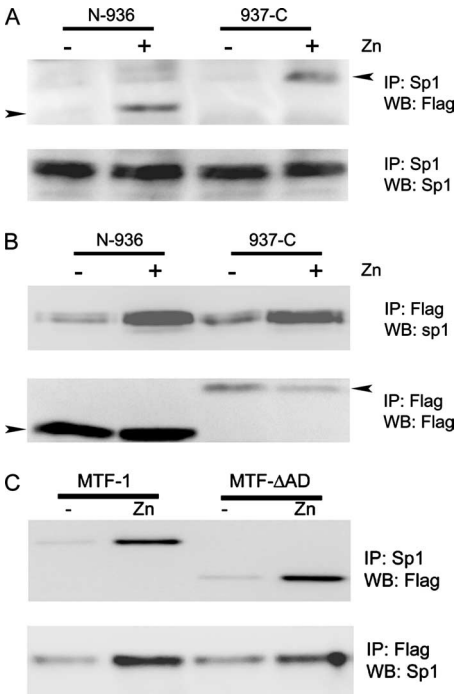

FIG. 6. Zinc-dependent binding of Sp1 involves both N- and C-terminal regions of MTF-1. MEF cells that lack MTF-1 were stably transfected with expression vectors (see Fig. 5 for vector details) encoding the N-terminal region of MTF-1 (N-936), the C-terminal region of MTF-1 (937-C) (A and B), MTF-ΔAD, or MTF-1_{flag} (MTF-1) (C). Transfected cells were incubated with (+) or without (−) 100 μM $ZnSO_4$ (Zn) for 1 h, and whole-cell extracts were reciprocally coimmunoprecipitated (IP) and Western blotted (WB) using the Sp1 and Flag antibodies as indicated.

MTF-1 in recruitment of p300 is reminiscent of the role of an acidic transactivation domain in HIF-1α, which has been shown to directly interact through hydrophobic and polar residues with the cysteine- and histidine-rich 1 (CH1) domain of p300 (9, 14). Although there is no evidence for direct interactions between MTF-1 and p300, the effect of specifically mutating several leucine residues in the MTF-1 acidic domain was examined (Fig. 8A). Seven of the eight total leucine residues in the acidic domain were found near the beginning of this domain (L335, L337, L340, L342, L343, L349, and L361), and each of these was mutated to alanine (Fig. 8A). Interestingly, these leucine residues are clustered within or very near a previously described nuclear exclusion signal peptide (33). This entire nuclear exclusion peptide was deleted when the acidic domain was removed from MTF-1.

Northern blotting revealed that mutation of these seven leucine residues abolished MTF-1 induction of *MT-I* expression in response to zinc (Fig. 8B). Coimmunoprecipitation experiments demonstrated that these mutations significantly decreased (~8-fold) the recruitment of p300 to the MTF-1 complex (Fig. 8C) but did not impair zinc-dependent MRE-binding activity in an EMSA (Fig. 8D), in agreement with previously published results (33). As mentioned above, five of the seven mutated leucine residues are located within a nuclear exclusion signal in MTF-1. As anticipated, relative to wild-type MTF-1, the MTF-1 with leucine mutations mainly localized in the nucleus in the absence of zinc treatment (Fig. 8E). In this Western blot, the relative amounts of MTF-1 in the cytosol and nuclear extracts are not adjusted on a per-cell basis. One should compare the signals detected in the nuclear extracts to one another to judge nuclear localization. There is no increase in the amount of MTF-1 in the cell in response to zinc in these experiments (data not shown). Taking these data together, we conclude that these leucine residues in the acidic domain play a critical role in the transactivation activity of MTF-1 as well as in the formation of a zinc-dependent MTF-1 complex containing p300.

Zinc does not bind to the acidic domain of MTF-1. Given the existence of zinc-dependent steps in the nuclear localization, p300 recruitment, and *MT-I* transcriptional activation functions involving the MTF-1 acidic region (Fig. 7 and 8), NMR experiments were carried out to characterize its structure in the presence of zinc (Fig. 9). A recombinant peptide extending from zf6 to the acidic domain was purified and analyzed. NMR chemical shift comparisons of backbone $^{13}C\alpha$ shifts in a zf6-acidic domain peptide to those of corresponding amino acids in random coil peptides (42, 43) (Fig. 9A) clearly show that, in the presence of 1 molar equivalent of zinc, zf6 adopts a canonical ββα zinc finger fold (22). In contrast, with the exception of four isolated amino acids (three of which directly precede proline residues), all of the $^{13}C\alpha$ chemical shift differences in the acidic region are less than 0.75 ppm. This demonstrates that the acidic domain of MTF-1 lacks defined secondary structure in the majority of solution conformers. An essentially identical structure in the acidic domain of MTF-1 is adopted in the presence of 3 molar equivalents of zinc (Fig. 9B), and nearly identical NMR backbone $^{13}C\alpha$ chemical shifts were observed for the MTF-1 acidic region in the absence of added zinc, while the zf6 region predictably loses the metal-dependent ββα fold (data not shown). These results indicate an absence of zinc binding directly to the acidic domain and suggest that the formation of a zinc-dependent complex containing MTF-1 involves a more complicated process than structural changes within the acidic region induced by zinc coordination.

DISCUSSION

A recent study of *Drosophila* MTF-1, which mediates copper responsiveness of the fly *MT* genes, demonstrated that the coactivator complexes TFIID and Mediator interact functionally to modulate metal induction. *Drosophila* MTF-1 recruits TFIID, which then binds to promoter DNA. The initiation of transcription is subsequently activated by recruitment of the mediator complex (27). *Drosophila* MTF-1 was shown to require different coactivator subunits, depending on the context of the core promoter (27). Our studies extend those observations by demonstrating that mouse MTF-1 also forms a multiprotein complex containing p300/CBP and Sp1 in response to zinc or cadmium.

Although p300 and CBP share high degrees of homology and similar expression patterns, they can play unique and distinct roles in gene regulation (15). CBP and p300 both form complexes with MTF-1 in response to zinc. Our data demonstrate that p300 plays a role in the activation of the *MT-I* gene, but we cannot exclude the possibility that residual CBP present after RNAi treatment is sufficient for this function. In contrast,

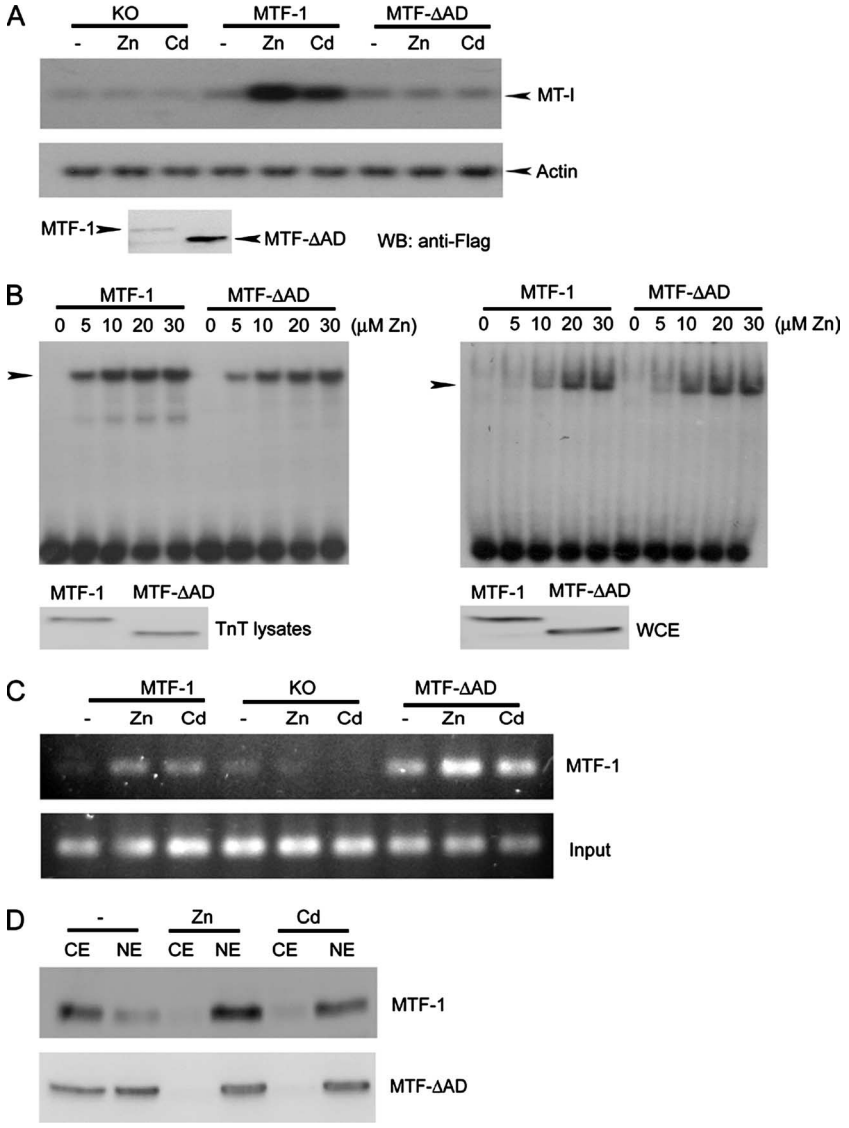

FIG. 7. Analyses of the effects of deletion of the acidic transactivation domain of MTF-1 on nuclear translocation, *MT-I* promoter binding, and *MT-I* expression in response to zinc or cadmium. (A) Northern blot analysis of *MT-I* mRNA in MEF cells that lack MTF-1 (KO) or were transfected with $MTF\text{-}1_{flag}$ (MTF-1) or MTF-ΔAD expression vectors (see Fig. 5 for vector details). Cells were incubated for 6 h in medium with or without (−) 100 μM $ZnSO_4$ (Zn) or 10 μM $CdCl_2$ (Cd). β-Actin mRNA served as a control (middle). The relative amounts of MTF-1 and MTF-ΔAD peptides were monitored by Western blot analysis (WB) using an anti-Flag antibody (lower panel). (B) EMSA analysis of the zinc-dependent MRE-binding activities of MTF-1 and MTF-ΔAD synthesized in vitro (left panels) or expressed in vivo in transfected cells (right panels). TnT lysates or whole-cell extracts (WCE) prepared from transfected cells were adjusted to the indicated concentrations of zinc (0 to 30 μM), and MRE-binding activity was measured, as detailed in Materials and Methods. The arrow demarcates the specific MTF-1–MRE–zinc complex. The relative amounts of MTF-1 and MTF-ΔAD in the EMSA were monitored by Western blotting using a Flag antibody (bottom panels). (C) ChIP analysis of the binding of MTF-1 and MTF-ΔAD to the *MT-I* promoter in response to zinc or cadmium. Transfected cells were incubated in medium containing Zn or Cd for 1 h, and chromatin was immunoprecipitated using a Flag antibody. The relative amount of *MT-I* promoter DNA in the immunoprecipitate was determined by PCR. Uniformity in the input DNA was monitored by PCR (bottom). (D) Western blot detection of MTF-1 and MTF-ΔAD in cytoplasmic (CE) and nuclear (NE) extracts from transfected cells before (−) or after incubation for 1 h in medium containing Zn or Cd.

neither CBP nor p300 apparently plays a critical role in MTF-1 induction of the *ZnT1* gene in response to zinc. These findings support the notion that, similar to *Drosophila* MTF-1, mouse MTF-1 must require different coactivator complexes, depending on the context of the core promoter and perhaps the inducer. Since many different genes are regulated by MTF-1, CBP may be involved in the regulation of target genes that were not examined.

How MTF-1 facilitates the recruitment of p300 or Sp1 to a zinc-dependent complex remains to be determined. The acidic domain of MTF-1 is required, and several leucine residues within that domain are important for p300 recruitment to the complex. On the surface, there are several similarities between the acidic domain of HIF-1α and that of MTF-1. Both are rich in acidic amino acids and have critical leucine residues that are important for the ability to transactivate gene expression via

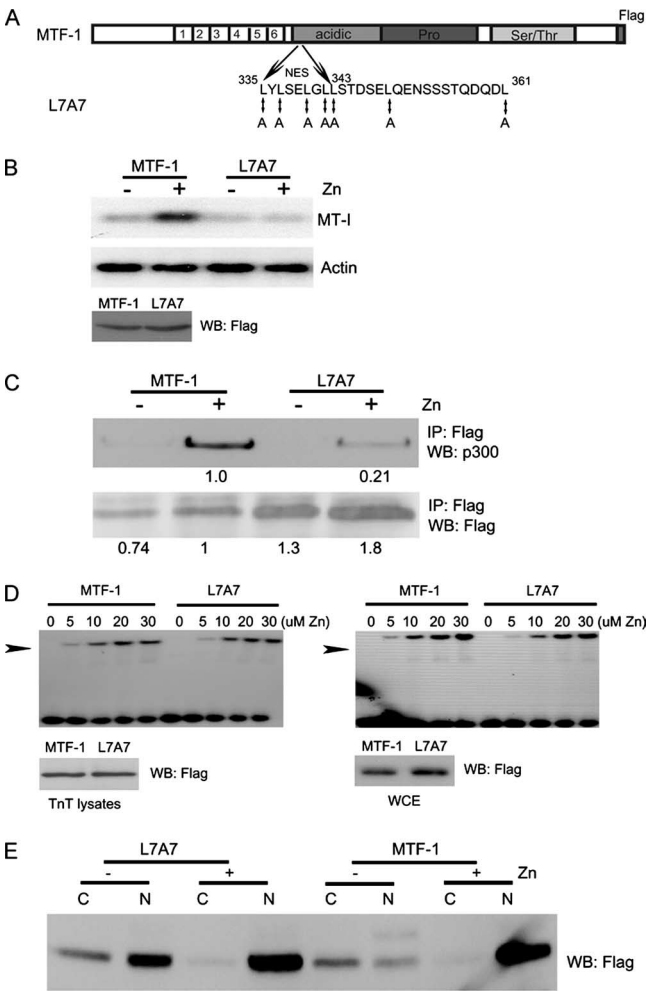

FIG. 8. Alanine-for-leucine substitutions in the MTF-1 acidic domain abrogate its induction of *MT-I* expression and formation of a complex with p300 but promote nuclear localization. (A) Seven leucine residues in the acidic transactivation domain of MTF-1 were mutated to alanine (L7A7). Five of these leucines (residues 335, 337, 310, 312, and 313) are within a previously identified nuclear exclusion signal sequence (33). (B) Cells stably transfected with expression vectors for $MTF\text{-}1_{flag}$ or MTF_{flag} with seven leucine-to-alanine substitutions (L7A7) in the acidic domain were incubated with 100 μM $ZnSO_4$ (Zn) for 6 h, and *MT-I* mRNA (upper) and β-*actin* mRNA (middle) were detected by Northern blotting. Levels of MTF-1 protein were determined by Western blotting using a Flag antibody (lower). (C) Stably transfected cells were incubated with 100 μM $ZnSO_4$ (Zn) for 1 h, and a coimmunoprecipitation (IP) assay was performed using Flag and p300 antibodies as indicated (see legend to Fig. 2). WB, Western blotting. (D) The zinc-dependent DNA-binding activities of $MTF\text{-}1_{flag}$ and the MTF-1 mutant (L7A7) were examined by EMSA. TnT lysates or whole-cell extracts (WCE) prepared from transfected cells were adjusted to the indicated concentrations of zinc (0 to 30 μM), and MRE-binding activity was measured, as detailed in Materials and Methods. The arrow demarcates the specific MTF-1–MRE–zinc complex. The relative amounts of $MTF\text{-}1_{flag}$ and the MTF-1 mutant (L7A7) in the EMSA were monitored by Western blotting using a Flag antibody (bottom panels). (E) Cytoplasmic (C) and nuclear (N) extracts were prepared from stably transfected cells before (−) or after (+) a 1-h incubation with 100 μM $ZnSO_4$ (Zn). Extracts were analyzed by Western blotting using a Flag antibody. The amount of cytoplasmic extract was not normalized to the amount of nuclear extract. One should compare the signals in the nuclear extract lanes to judge nuclear localization before and after zinc treatment relatively.

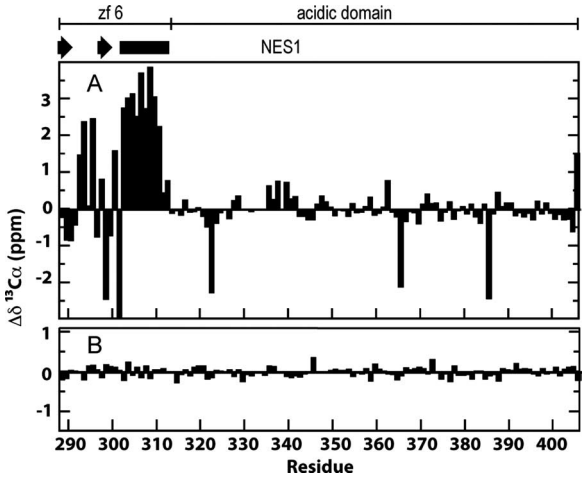

FIG. 9. The isolated zf6-acidic domain region of MTF-1 lacks regular secondary structure in the presence of 1 and 3 molar equivalents of zinc. (A) Bar plot of backbone $^{13}C\alpha$ NMR chemical shift differences between residues from the zf6-acidic domain protein in the presence of 1 molar equivalent of zinc and those like amino acids from small, unstructured random coil peptides (42, 43). This comparison is a sensitive measure of secondary protein structure. The secondary structure of zf6-acidic domain protein (NES1) is indicated above the bar plot, using block arrows and a rectangle to represent those residues in β-strand and helical conformations, respectively. (B) Bar plot of backbone $^{13}C\alpha$ chemical shift differences between zf6-acidic domain proteins in the presence of 3.3 and 1.1 molar equivalents of zinc.

p300. The HIF-1α acidic domain adopts a conformation with α helices that are leucine rich, which binds to the CH1 domain of p300 by hydrophobic interactions as well as by polar interactions in a positively charged pocket (9). The presence of several histidine residues in the acidic domain of MTF-1 suggested that zinc might bind to this region. However, NMR chemical shift analyses reveal that the acidic domain lacks secondary structure in the presence or absence of zinc, which indicates that zinc has no direct interaction with or effects on the conformation of the MTF-1 acidic domain in the absence of interaction with other factors. An alternative origin of the zinc-dependent MTF-1–p300 interaction could involve zinc binding to p300. Indeed, the CH1 domain of p300 contains three zinc-binding clusters of HCCC-type coordination that form structure in the absence of binding partners (12). This domain of p300 binds a C-terminal transactivation domain of HIF-1α (9, 14). The structure of the CH2 domain is also stabilized by zinc and interacts with the N terminus of p53 (11). Further experiments will be performed to investigate whether zinc can change the conformation of p300 to facilitate its binding to MTF-1.

One of the interesting findings in this study was that leucine residues in the acidic domain of MTF-1, which are clustered in and around a previously described nuclear exclusion signal (33), are also critical for the recruitment of p300 and the formation of a functional coactivator complex. In previous studies, it was shown that leucine-to-alanine substitutions in the nuclear exclusion signal caused MTF-1 to accumulate in the nucleus and eliminated zinc-induced activation of *MT-I* gene expression (33). Our studies confirm and extend those findings by showing that these leucine residues are important for zinc-induced formation of the MTF-1 complex containing

p300. Since p300 was found to play an important role in regulating *MT-I* gene expression in response to zinc, our studies suggest a model in which there is a competition between formation of a transcription factor complex and nuclear export of MTF-1, and this equilibrium is controlled by the availability of zinc within the cell.

The recruitment of Sp1 to an MTF-1 complex does not require the acidic transactivation domain of MTF-1 yet is still zinc dependent. Sp1 was recruited to the amino-terminal half of MTF-1 as well as to the C-terminal half of MTF-1. The latter likely reflects, in part, the fact that p300 can also bind Sp1 (37), and that complex may be recruited to the acidic domain of MTF-1 in response to zinc. However, Sp1 was also recruited in the absence of the acidic domain by as-yet-unknown mechanisms. Our data suggest that there are two potential binding sites for Sp1 or that Sp1 is corecruited to other regions of MTF-1 by other, unidentified factors. Elucidation of those mechanisms requires further studies.

Whether Sp1 recruitment is important for *MT-I* gene regulation remains to be determined. A potential Sp1 binding site overlaps MRE-d in the mouse *MT-I* promoter (36), and an interaction of Sp1 with MTF-1 has been suggested by MRE selection studies (40). In addition, it has been suggested that competition between Sp1 and MTF-1 may affect activation of gene expression (30). However, Sp1 can repress or activate transcription, depending on the promoter and on posttranslational modifications of the protein itself (23). Herein, we establish that Sp1 is recruited to an MTF-1 complex in response to zinc.

It is interesting to note that p300 can bind Sp1 and then acetylate its DNA-binding domain (37). The evidence reported herein establishes that MTF-1 facilitates the association of Sp1 and p300 in a zinc-dependent complex. Phorbol ester-induction of the 12(*S*)-lipoxygenase gene induces the formation of a c-Jun–p300–Sp1 complex. The Sp1–c-Jun complex recruits histone deacetylase 1 (HDAC1) to the promoter and silences this gene, while Sp1 becomes acetylated. Phorbol ester treatment causes the disassociation of HDAC, the deacetylation of Sp1, and the recruitment of p300 (17). Similarly, inhibition of HDAC by trichostatin A can activate the *TGFβRII* promoter, which is accompanied by changes in an Sp1–NF-Y–p300–PCAF–HDAC complex (16). Inhibition of HDAC led to the recruitment of p300 into the complex and the acetylation of Sp1. We have noted that basal expression of the mouse *MT-I* gene is unaffected following a short (several-hour) exposure to tryptic soy agar (G. K. Andrews, unpublished observation), which suggests that these genes in MEF cells are not repressed by HDAC activity. Therefore, we assume that Sp1 most likely acts as a positive regulator of *MT-I* gene expression.

In summary, results reported herein demonstrate that mouse MTF-1 forms a multiprotein complex containing p300/CBP and Sp1 in response to zinc or cadmium. MTF-1 independently facilitates the incorporation of p300 and Sp1 into this complex, and p300 plays a crucial role in the zinc-induced *MT-I* gene expression but not in induction of *ZnT1*.

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REFERENCES

1. **Adilakshmi, T., and R. O. Laine.** 2002. Ribosomal protein S25 mRNA partners with MTF-1 and La to provide a p53-mediated mechanism for survival or death. J. Biol. Chem. **277:**4147–4151.
2. **Andrews, G. K.** 2000. Regulation of metallothionein gene expression by oxidative stress and metal ions. Biochem. Pharmacol. **59:**95–104.
3. **Andrews, G. K.** 2001. Cellular zinc sensors: MTF-1 regulation of gene expression. Biometals **14:**223–237.
4. **Andrews, G. K., D. K. Lee, R. Ravindra, P. Lichtlen, M. Sirito, M. Sawadogo, and W. Schaffner.** 2001. The transcription factors MTF-1 and USF1 cooperate to regulate mouse metallothionein-I expression in response to the essential metal zinc in visceral endoderm cells during early development. EMBO J. **20:**1114–1122.
5. **Bittel, D., T. Dalton, S. Samson, L. Gedamu, and G. K. Andrews.** 1998. The DNA-binding activity of metal response element-binding transcription factor-1 is activated in vivo and in vitro by zinc, but not by other transition metals. J. Biol. Chem. **273:**7127–7133.
6. **Chan, H. M., and N. B. La Thangue.** 2001. p300/CBP proteins: HATs for transcriptional bridges and scaffolds. J. Cell Sci. **114:**2363–2373.
7. **Chen, X. H., B. Zhang, P. M. Harmon, W. Schaffner, D. O. Peterson, and D. P. Giedroc.** 2004. A novel cysteine cluster in human metal-responsive transcription factor 1 is required for heavy metal-induced transcriptional activation *in vivo*. J. Biol. Chem. **279:**4515–4522.
8. **Cramer, M., I. Nagy, B. J. Murphy, M. Gassmann, M. O. Hottiger, O. Georgiev, and W. Schaffner.** 2005. NF-kappaB contributes to transcription of placenta growth factor and interacts with metal responsive transcription factor-1 in hypoxic human cells. Biol. Chem. **386:**865–872.
9. **Dames, S. A., M. Martinez-Yamout, R. N. De Guzman, H. J. Dyson, and P. E. Wright.** 2002. Structural basis for Hif-1 alpha /CBP recognition in the cellular hypoxic response. Proc. Natl. Acad. Sci. USA **99:**5271–5276.
10. **Daniels, P. J., and G. K. Andrews.** 2003. Dynamics of the metal-dependent transcription factor complex *in vivo* at the mouse metallothionein-I promoter. Nucleic Acids Res. **31:**6710–6721.
11. **De Guzman, R. N., H. Y. Liu, M. Martinez-Yamout, H. J. Dyson, and P. E. Wright.** 2000. Solution structure of the TAZ2 (CH3) domain of the transcriptional adaptor protein CBP. J. Mol. Biol. **303:**243–253.
12. **De Guzman, R. N., J. M. Wojciak, M. A. Martinez-Yamout, H. J. Dyson, and P. E. Wright.** 2005. CBP/p300 TAZ1 domain forms a structured scaffold for ligand binding. Biochemistry **44:**490–497.
13. **Delaglio, F., S. Grzesiek, G. W. Vuister, G. Zhu, J. Pfeifer, and A. Bax.** 1995. NMRPipe: a multidimensional spectral processing system based on UNIX pipes. J. Biomol. NMR **6:**277–293.
14. **Freedman, S. J., Z. Y. Sun, F. Poy, A. L. Kung, D. M. Livingston, G. Wagner, and M. J. Eck.** 2002. Structural basis for recruitment of CBP/p300 by hypoxia-inducible factor-1 alpha. Proc. Natl. Acad. Sci. USA **99:**5367–5372.
15. **Goodman, R. H., and S. Smolik.** 2000. CBP/p300 in cell growth, transformation, and development. Genes Dev. **14:**1553–1577.
16. **Huang, W., S. Zhao, S. Ammanamanchi, M. Brattain, K. Venkatasubbarao, and J. W. Freeman.** 2005. Trichostatin A induces transforming growth factor beta type II receptor promoter activity and acetylation of Sp1 by recruitment of PCAF/p300 to a Sp1.NF-Y complex. J. Biol. Chem. **280:**10047–10054.
17. **Hung, J. J., Y. T. Wang, and W. C. Chang.** 2006. Sp1 deacetylation induced by phorbol ester recruits p300 to activate 12(*S*)-lipoxygenase gene transcription. Mol. Cell. Biol. **26:**1770–1785.
18. **Jiang, H. M., P. J. Daniels, and G. K. Andrews.** 2003. Putative zinc-sensing zinc fingers of metal-response element-binding transcription factor-1 stabilize a metal-dependent chromatin complex on the endogenous metallothionein-I promoter. J. Biol. Chem. **278:**30394–30402.
19. **Johnson, B. A., and R. A. Blevins.** 1994. NMR View: a computer program for the visualization and analysis of NMR data. J. Biomol. NMR **4:**603–614.
20. **Laity, J. H., and G. K. Andrews.** 2007. Understanding the mechanisms of zinc-sensing by metal-response element binding transcription factor (MTF-1). Arch. Biochem. Biophys. **463:**201–210.
21. **Langmade, S. J., R. Ravindra, P. J. Daniels, and G. K. Andrews.** 2000. The transcription factor MTF-1 mediates metal regulation of the mouse ZnT1 gene. J. Biol. Chem. **275:**34803–34809.
22. **Lee, M. S., G. P. Gippert, K. V. Soman, D. A. Case, and P. E. Wright.** 1989. Three-dimensional solution structure of a single zinc finger DNA-binding domain. Science **245:**635–637.
23. **Li, L., S. He, J. M. Sun, and J. R. Davie.** 2004. Gene regulation by Sp1 and Sp3. Biochem. Cell Biol. **82:**460–471.
24. **Li, Y., T. Kimura, J. H. Laity, and G. K. Andrews.** 2006. The zinc-sensing mechanism of mouse MTF-1 involves linker peptides between the zinc fingers. Mol. Cell. Biol. **26:**5580–5587.
25. **Lichtlen, P., and W. Schaffner.** 2001. Putting its fingers on stressful situations: the heavy metal-regulatory transcription factor MTF-1. Bioessays **23:**1010–1017.
26. **Lichtlen, P., Y. Wang, T. Belser, O. Georgiev, U. Certa, R. Sack, and W.**

Schaffner. 2001. Target gene search for the metal-responsive transcription factor MTF-1. Nucleic Acids Res. **29:**1514–1523.
27. **Marr, M. T., Y. Isogai, K. J. Wright, and R. Tjian.** 2006. Coactivator cross-talk specifies transcriptional output. Genes Dev. **20:**1458–1469.
28. **Murphy, B. J., T. Kimura, B. G. Sato, Y. Shi, and G. K. Andrews.** 2008. Metallothionein induction by hypoxia involves cooperative interactions between MTF-1 and HIF-1a. Mol. Cancer Res. **6:**483–490.
29. **Murphy, B. J., B. G. Sato, T. P. Dalton, and K. R. Laderoute.** 2005. The metal-responsive transcription factor-1 contributes to HIF-1 activation during hypoxic stress. Biochem. Biophys. Res. Commun. **337:**860–867.
30. **Ogra, Y., K. Suzuki, P. Gong, F. Otsuka, and S. Koizumi.** 2001. Negative regulatory role of Sp1 in metal responsive element-mediated transcriptional activation. J. Biol. Chem. **276:**16534–16539.
31. **Potter, B. M., L. S. Feng, P. Parasuram, V. A. Matskevich, J. A. Wilson, G. K. Andrews, and J. H. Laity.** 2005. The six zinc fingers of metal-responsive element binding transcription factor-1 form stable and quasi-ordered structures with relatively small differences in zinc affinities. J. Biol. Chem. **280:** 28529–28540.
32. **Potter, B. M., N. A. Knudsen, L. S. Feng, V. A. Matskevich, J. A. Wilson, G. K. Andrews, and J. H. Laity.** 2005. NMR assignment of the six zinc fingers of MTF-1 in the free and DNA-bound states. J. Biomol. NMR **32:**94.
33. **Saydam, N., O. Georgiev, M. Y. Nakano, U. F. Greber, and W. Schaffner.** 2001. Nucleo-cytoplasmic trafficking of metal-regulatory transcription factor 1 is regulated by diverse stress signals. J. Biol. Chem. **276:**25487–25495.
34. **Saydam, N., F. Steiner, O. Georgiev, and W. Schaffner.** 2003. Heat and heavy metal stress synergize to mediate transcriptional hyperactivation by metal-responsive transcription factor MTF-1. J. Biol. Chem. **278:**31879–31883.
35. **Smirnova, I. V., D. C. Bittel, R. Ravindra, H. Jiang, and G. K. Andrews.** 2000. Zinc and cadmium can promote the rapid nuclear translocation of MTF-1. J. Biol. Chem. **275:**9377–9384.
36. **Stuart, G. W., P. F. Searle, and R. D. Palmiter.** 1985. Identification of multiple metal regulatory elements in mouse metallothionein-I promoter by assaying synthetic sequences. Nature **317:**828–831.
37. **Suzuki, T., A. Kimura, R. Nagai, and M. Horikoshi.** 2000. Regulation of interaction of the acetyltransferase region of p300 and the DNA-binding domain of Sp1 on and through DNA binding. Genes Cells **5:**29–41.
38. **Uenishi, R., P. Gong, K. Suzuki, and S. Koizumi.** 2006. Cross talk of heat shock and heavy metal regulatory pathways. Biochem. Biophys. Res. Commun. **341:**1072–1077.
39. **Vo, N., and R. H. Goodman.** 2001. CREB-binding protein and p300 in transcriptional regulation. J. Biol. Chem. **276:**13505–13508.
40. **Wang, Y., I. Lorenzi, O. Georgiev, and W. Schaffner.** 2004. Metal-responsive transcription factor-1 (MTF-1) selects different types of metal response elements at low vs. high zinc concentration. Biol. Chem. **385:**623–632.
41. **Wang, Y., U. Wimmer, P. Lichtlen, D. Inderbitzin, B. Stieger, P. J. Meier, L. Hunziker, T. Stallmach, R. Forrer, T. Rulicke, O. Georgiev, and W. Schaffner.** 2004. Metal-responsive transcription factor-1 (MTF-1) is essential for embryonic liver development and heavy metal detoxification in the adult liver. FASEB J. **18:**1071–1079.
42. **Wishart, D. S., B. D. Sykes, and F. M. Richards.** 1991. Relationship between nuclear magnetic resonance chemical shift and protein secondary structure. J. Mol. Biol. **222:**311–333.
43. **Wishart, D. S., B. D. Sykes, and F. M. Richards.** 1991. Simple techniques for the quantification of protein secondary structure by 1H NMR spectroscopy. FEBS Lett. **293:**72–80.